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The Kinetics of Chlorite and Chlorate in the Rat

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ABSTRACT

Chlorine dioxide (ClO_2) is under consideration as an alternative to chlorination as a disinfectant for public water supplies. The primary products resulting from ClO_2 disinfection of surface waters are chlorite (ClO_2^-) and chlorates (ClO_3^-). The kinetics of $^{36}ClO_2^-$ and $^{36}ClO_3^-$ was studied in rats. Radioactivity was rapidly absorbed from the gastrointestinal tract following the administration of (0.17 μCi) $^{36}ClO_2^-$ or (0.85 μCi) $^{36}ClO_3^-$ orally, and ^{36}Cl in plasma reached a peak at 2 hours and 1 hour, respectively. After 72 hours, radioactivity was highest in whole blood, followed by packed cells, plasma, stomach, testes, skin, lung, kidney, duodenum, carcass, spleen, ileum, brain, bone marrow, and liver in $^{36}ClO_2^-$ treatment. ^{36}Cl excretion was greatest at 24 hours after the administration of $^{36}ClO_3^-$, but in the $^{36}ClO_2^-$, the excretion most likely represented saturation of the biotransformation and excretion pathways. About 40% of the total initial dose was excreted at 72 hours in the urine and feces in both treatments. No ^{36}Cl was detected in expired air throughout the 72 hours studied.

INTRODUCTION

INDUSTRIAL wastes, domestic sewage, and agricultural runoff all contribute to the problem of water pollution by organic chemicals. Recent studies have demonstrated that the interaction of chlorine with various organic substances in the water results in the formation of halogenated compounds, such as chloroform, bromodichloromethane, dibromochloromethane, and bromoform (Rook, 1976). The awareness of the widespread occurrence of this type of pollution is due, in large part, to recent development in techniques for the identification of trace organic contaminants. Bellar et al. (1974) found that the concentration of trihalomethanes increased each time chlorine was added within the water treatment scheme.

Therefore, the use of chlorine is to be limited and the possible use of other disinfectants promoted. Among them is chlorine dioxide (ClO_2), which does not form trihalomethanes in drinking water (Miltner, 1976). However, the primary products resulting from ClO_2 disinfection of surface waters include chlorites and chlorates, which appear in concentrations 50% and 30% of ClO_2 demand, respectively (Miltner, 1976). Metabolism studies revealed that ClO_2 is converted to chloride, chlorite and chlorate in the rat (Abdel-Rahman et al., 1979b).

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It is important to consider the health effects of ingesting chlorite (ClO_2^-) and chlorate (ClO_3^-), the primary products resulting from ClO_2 disinfection of surface water. The oral administration of chlorates has been shown to produce methemoglobinemia (Methb) with blood destruction (Richardson, 1937). Jung and Kuon (1951) reported that the rate of Methb formation by chlorate was nearly proportional to the square root of the chlorate concentration.

Chlorite is thought to oxidize hemoglobin more rapidly than chlorate (Heubner and Jung, 1941). Hopf (1965) reported that the toxicity of chlorite might be similar to that of chlorate and indicated that subjects sensed a weak furry feeling after drinking sodium chlorite at a concentration of more than 0.3 mg/L. Musil et al. (1964) demonstrated chlorite to be a powerful producer of Methb in rats and recommended that water for consumption contain no ClO_2^-, since it might prove toxic to neonates. Heffernan et al. (1979) and Abdel-Rahman et al. (1979a) reported that ClO_2^- induced decreases in glutathione in vivo, which was accompanied by an increase in hydrogen peroxide. Hemolytic anemia occurred as noted by an increased turnover of red cells in cats exposed to ClO_2^- orally. There were few other signs of chlorite toxicity observed. At 500 mg ClO_2^-/L a significant increase in kidney/body weight ratio was observed, which may indicate some renal toxicity (Heffernan et al., 1979). In previous studies we reported that rats drinking ClO_2^- and ClO_3^- daily for 9 months exhibited depressed red blood cell counts, hemoglobin concentration, and packed cell volumes. Also, ^{3}H-thymidine incorporation studies revealed that both compounds diminished DNA synthesis in liver and testes after 3 months treatment (Abdel-Rahman et al., 1984).

The studies described in this report were conducted to provide information on the absorption, distribution, elimination, and metabolism of chlorite and chlorate in drinking water.

MATERIALS AND METHODS

Since the radioisotope chlorite and chlorate using ^{36}Cl are not commercially available, it was necessary to produce this material from the commercially available ^{36}Cl-labeled HCl (New England Nuclear).

The Synthesis of $K^{36}ClO_3$

The $K^{36}ClO_3$ was synthesized according to the method of Abdel-Rahman et al. (1979b). Part of $K^{36}ClO_3$ was used for the kinetics of chlorate, and the other part was used for generation of chlorine dioxide. Chlorine dioxide was then converted to chlorite by the addition of potassium iodide.

$$2^{36}ClO_2 + 2I^- \rightarrow 2^{36}ClO_2^- + I_2$$

Chlorite and Chlorate Absorption, Distribution, and Excretion Studies

A group of 4 male Sprague-Dawley rats (220–250 g) was administered 10 mg/L $^{36}ClO_2^-$ (0.17 μCi) orally. In another set of experiments another group received 5 mg/L $^{36}ClO_3^-$ (0.85 μCi) orally. Blood samples (heparinized) were collected at 5, 10, 20, 30, and 60 minutes and 2, 4, 8, 24, and 48 hours by orbital sinus puncture. The blood was centrifuged at 1000 × *g* for 15 minutes to separate the red blood cells from the plasma. At 72 hours rats were killed by decapitation, and blood was collected in heparinized tubes. Tissue specimens of stomach, testes, lung, kidney, duodenum, ileum, spleen, liver, bone marrow, carcass, and skin were prepared for the determination of ^{36}Cl content by liquid scintillation spectrometry, as described in our previous report (Abdel-Rahman et al., 1979a).

In another set of experiments, identical groups received orally the same concentrations and specific activity of chlorite and chlorate. These animals were housed in modified Roth all glass metabolism chambers for the collection of expired air, fecal and urine samples at 8, 16, 24, 48, and 72 hours. Radioactivity of Cl^-, ClO_2^-, and ClO_3^- was then measured as described in a previous report (Abdel-Rahman et al., 1979b).

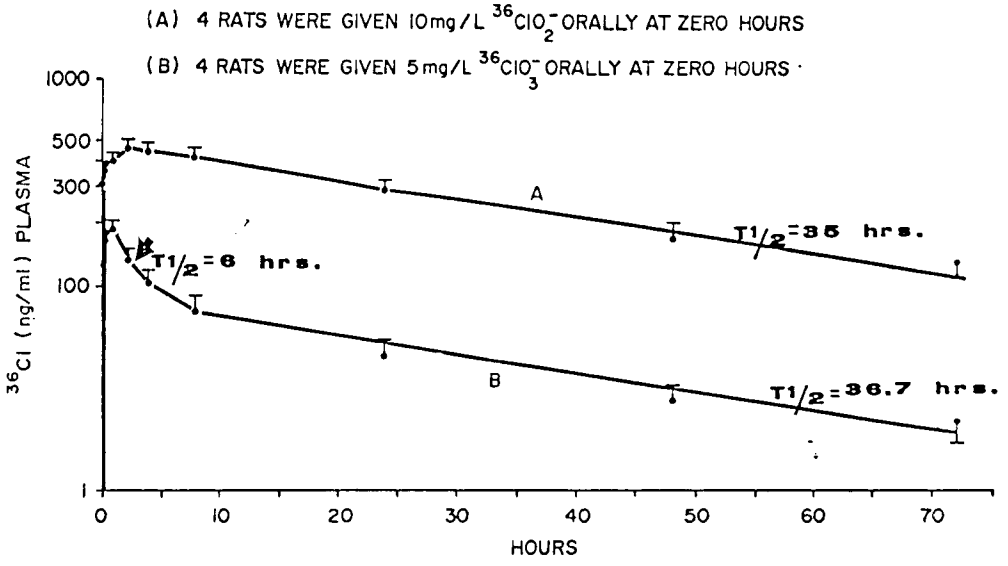

FIG. 1. Time course of $^{36}ClO_2^-$ and $^{36}ClO_3^-$ elimination from rat plasma.

RESULTS

Chlorite and Chlorate Absorption and Elimination from Blood

The absorption and elimination time course for chlorite and chlorate in rat plasma are shown in Figure 1. A peak ^{36}Cl plasma level (470 ng/ml) was reached at 2 hours following oral administration of 3 ml of 10 mg/L $^{36}ClO_2^-$. The half-life for the elimination of ^{36}Cl from the rat was 35 hours, corresponding to a rate constant of 0.02 $hour^{-1}$ (Figure 1A). When rats drank 3 ml of 5 mg/L $^{36}ClO_3^-$, a peak ^{36}Cl plasma level (185 ng/ml) was reached at 30 minutes. The half-life for the rapid elimination from plasma was about 6 hours, with a rate constant 0.12 $hour^{-1}$, followed by a slower phase of elimination having a 36.7 hour half-life, corresponding to a rate constant of 0.018 $hour^{-1}$ (Figure 1B).

Chlorite and Chlorate Distribution

Figure 2 reveals that the distribution of ^{36}Cl compounds 72 hours after the administration of $^{36}ClO_2^-$ was the highest in whole blood, followed by packed cells, plasma, stomach, testes, skin, lung, kidney, duodenum, carcass, spleen, ileum, brain, bone marrow, and liver. However, in ClO_3^- treatment, radioactivity was highest in plasma, followed by whole blood, stomach, testes, lung, kidney, skin, duodenum, spleen, brain, packed cells, ileum, carcass, liver, and bone marrow (Figure 3).

Excretion and Metabolism Studies

Collections of urine, feces, and expired air were obtained over a 3-day period after the administration of chlorite and chlorate as described in Materials and Methods. The ^{36}Cl compounds, recovered from rats by pulmonary, urinary, and intestinal routes of excretion are summarized in Tables 1 and 2 for ClO_2^- and ClO_3^- treatments, respectively. Values are expressed as a percentage of the total initial doses.

After $^{36}ClO_2^-$ treatment, about 39% of ^{36}Cl was recovered in the 72-hour period. In the first 24

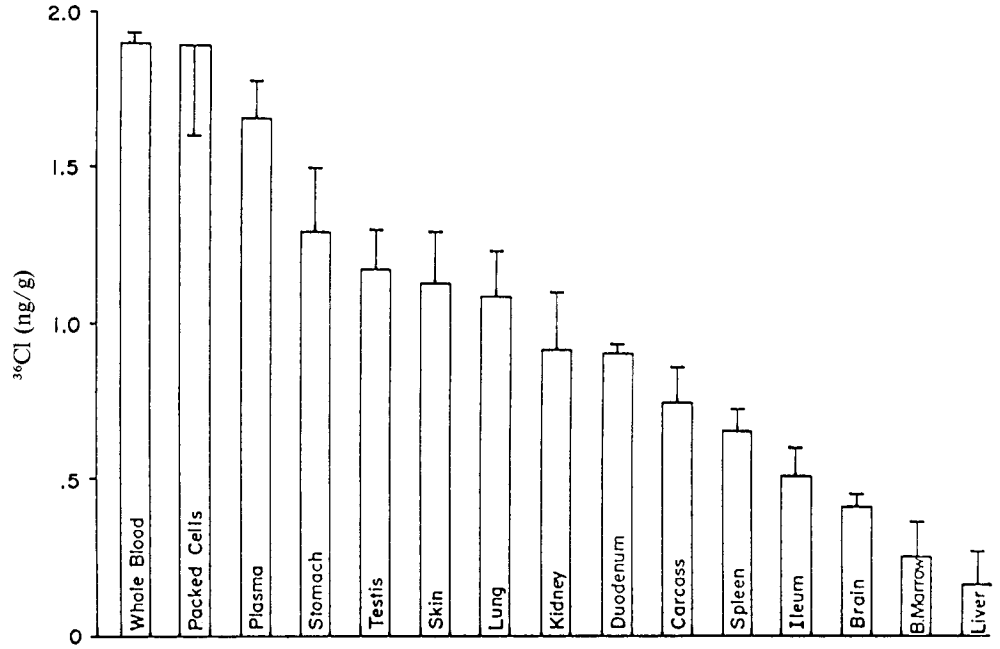

FIG. 2. $^{36}ClO_2^-$ distribution in rat.

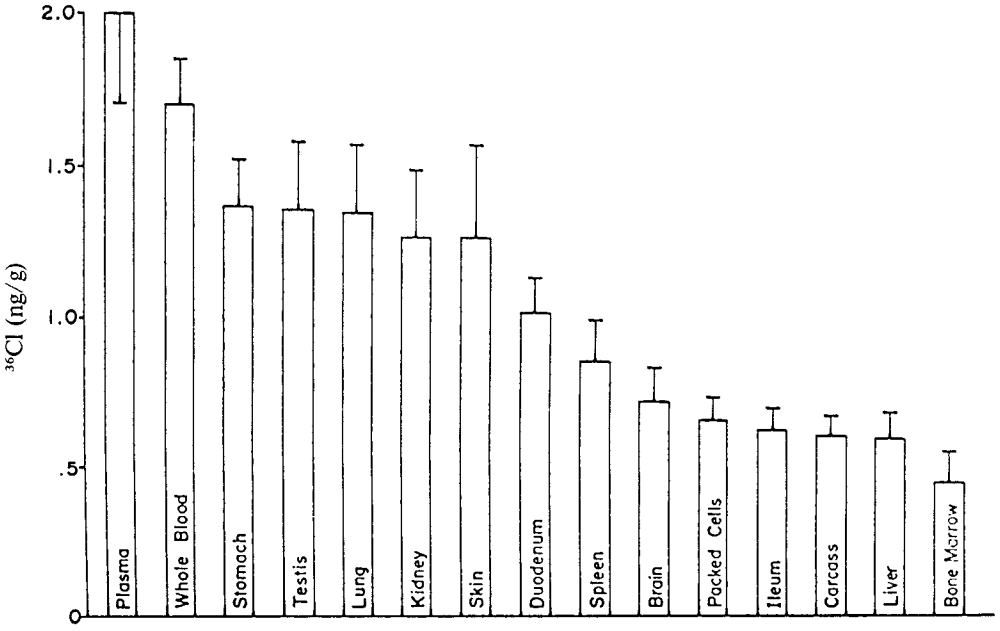

FIG. 3. $^{36}ClO_3^-$ distribution in rat.

TABLE 1. EXCRETION OF $^{36}ClO_2^-$ IN THE RAT[a]

Collection Period (hours)	Experiment No. 1			Experiment No. 2		
	Urine	Feces	Total	Urine	Feces	Total
0–8	4.00			2.00		
8–16	7.22			5.54		
16–24	3.57			5.32		
0–24	14.79	1.37	16.16	12.86	0.36	13.22
24–48	7.26	2.48	9.74	9.66	2.36	12.02
48–72	13.81	1.86	15.67	10.64	1.06	11.70
0–72	35.86	5.71	41.57	33.16	3.78	36.94

[a]Values represent data from 2 treated rats/experiment expressed as percentage of the total initial dose. ^{36}Cl was not detected in expired air throughout the 72 hours studied.

TABLE 2. EXCRETION OF $^{36}ClO_3^-$ IN THE RAT[a]

Collection Period (hours)	Experiment No. 1			Experiment No. 2		
	Urine	Feces	Total	Urine	Feces	Total
0–8	18.34			24.80		
8–16	3.80			8.66		
16–24	12.93			4.32		
0–24	35.07	3.92	38.99	37.78	1.02	38.80
24–48	1.03	0.02	1.05	0.93	0.58	1.51
48–72	1.92	0.16	2.08	3.54	0.58	4.12
0–72	38.02	4.10	42.12	42.25	2.18	44.43

[a]Values represent data from 2 treated rats/experiment expressed as percentage of the total initial dose. ^{36}Cl was not detected in expired air throughout the 72 hours studied.

TABLE 3. METABOLISM OF ClO_2^- IN RAT

	Collection Period (hours)	Analyte[a]	
		Cl^-	ClO_2^-
Urine			
	0–8	1.30 ± 0.30	1.00 ± 1.00
	8–16	6.15 ± 0.76	0.35 ± 0.35
	16–24	3.65 ± 1.10	0.45 ± 0.15
	24–48	8.65 ± 1.70	2.85 ± 2.00
	48–72	11.80 ± 0.71	1.40 ± 1.40

[a]Values represent the mean ± SE of data from 4 rats as percentage of the total initial dose. ClO_3^- was not detected throughout the 72 hours studied.

hours 14% was excreted in the urine, and 0.9% in feces. During the second 24 hours about 8% was obtained via urinary routes, while 2% was obtained in feces. At the end of the 3-day collection period, the total fractions of the initial dose eliminated by urinary routes was about 35%, while 5% was obtained in feces. ^{36}Cl was not detected in the expired air throughout the 72 hours studied.

The excretion after chlorate administration was about 43% in the 72-hour period. Most of ^{36}Cl (39%) was recovered via urinary and fecal routes during the first 24 hours. No radioactivity was detected in the expired air thoughout the 3-day collection period.

Metabolism studies revealed that $^{36}ClO_2^-$ is excreted as chloride and chlorite (Table 3). However, chlorate is eliminated as chloride, chlorite, and chlorate (Table 4).

TABLE 4. METABOLISM OF ClO_3^- IN RAT

Collection Period (hours)	Analyte[a]		
	Cl^-	ClO_2^-	ClO_3^-
0–8	5.45 ± 1.40	2.20 ± 2.20	11.7 ± 1.90
8–16	4.45 ● 2.50	0.05 ± 0.05	1.4 ± 0.10
16–24	7.30 ± 3.70	1.30 ± 0.61	0.10 ± 0.10
24–48	0.94 ± 0.04	0.005 ± 0.005	[b]
48–72	2.35 ± 0.66	0.35 ± 0.15	[b]

[a]Values represent the mean ± SE of data from 4 rats as percentage of the total initial dose.
[b]None detected.

DISCUSSION

The present data show that chlorate elimination from rat plasma occurs in 2 phases, α phase, which represents fast elimination ($t_{1/2}$ = 6 hours), and β phase, a slower elimination ($t_{1/2}$ = 36.7 hours). On the other hand, chlorite elimination most likely represents one phase of elimination.

The elimination of $^{36}ClO_2^-$ and $^{36}ClO_3^-$ from the body by kidney and intestine has been demonstrated. Although the major portion of the excretion in urine was detected as chloride, about 20% of the total initial dose was excreted as ClO_2^- thoughout the 3 days studied after $^{36}ClO_2^-$ administration. In the same time period, the excretion after $^{36}ClO_3^-$ administration was about 20.0, 4.0, and 13.0% as chloride, chlorite, and chlorate, respectively.

The distribution experiments indicated that the activity in the whole blood is equally distributed between plasma and packed cells after chlorite injection. However, a low activity in packed cells compared to plasma activity in chlorate ingestion was detected. The distribution studies reveal that ^{36}Cl compounds were high in stomach even 72-hours after the administration of $^{36}ClO_2^-$ and $^{36}ClO_3^-$ by gavage. The prolonged and high concentration of ClO_2^- and ClO_3^- metabolites in testes suggest possible pharmacological action at this site. Studies are currently in progress in our laboratory to study this phenomenon and further elucidate and characterize the potential toxication of these compounds in drinking water.

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